Some biochemical and genetic markers associated with borderline susceptibility (low-level resistance) to methicillin in *Staphylococcus aureus*.

Orietta Massidda, Maria Pia Montanari, Marina Mingoia and Pietro Emanuele Varaldo.
Istituto di Microbiologia, Universita' di Ancona, Ancona, Italy

Recently we reported that an inducible ß-lactamase, other than the classical penicillinase and capable of specifically hydrolyzing methicillin, was present in the membrane fraction of two clinical isolates of *Staphylococcus aureus* which showed low-level resistance to methicillin and related penicillins (penicillinase-resistant penicillins or PRPs). The study was extended to a larger number of *S. aureus* clinical isolates. Those able to hydrolyze methicillin were further analyzed. These strains also met the criteria for borderline susceptibility on the basis of their susceptibility levels to penicillins as previously proposed. The bacterial membranes of five *S. aureus* strains (one penicillin-resistant and fully susceptible to methicillin and four borderline) were analyzed by SDS-PAGE electrophoresis. Coomassie blue staining of the gels and analysis of ß-lactamase activity, by zymography of the renatured gels, showed that: (i) a band induced by methicillin (about 31 kDa) was detected in the membrane fraction of the borderline strains after staining the gel with coomassie blue; (ii) two bands (32 kDa and 31 kDa) showing ß-lactamase activity were detected in the membrane fraction of the borderline strains grown in the presence of methicillin, when nitrocefin or penicillin was used as substrate. The lower band was peculiar to the borderline strains. (iii) a single band showing ß-lactamase activity, presumably the 31 kDa, was detected in the membrane fraction of the borderline strains grown in the presence of methicillin, when PADAC or methicillin was used as substrate. Neither methicillin nor PADAC hydrolysis was detected in the membrane fraction of the susceptible strain.

Induction of ß-lactamase was studied in one of the borderline *S. aureus* strains. The bacterial membranes prepared from cells taken at various times after the addition of the inducer and assayed spectrophotometrically showed two peaks of ß-lactamase activity, one detected with nitrocefin and the second with PADAC. These data agree with our previous finding that two different ß-lactamase activities, with different substrate specificity, were present in the borderline *S. aureus* strains. DNA analysis showed that the borderline strains have also in common a plasmid of 17.2 kb, presumably pBW15, recently associated with the borderline phenotype in a widespread nosocomial infection in the United States. These results indicate that borderline susceptible *S. aureus* strains have more in common than just intermediate susceptibility levels to PRPs.